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Method for Determining Bioburden of Surgical Gloves

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A washing procedure that removed maximum numbers of contaminating microorganisms from whole surgical gloves was developed. Washing, coupled with membrane filtration, proved to be a simple and effective method for bioburden determinations on whole gloves.

Since the effective sterilization of a product depends on the number and type of organisms present (1), knowledge of the pre-sterilization bioload would allow shorter sterilization processing than if sterilization were based on an arbitrary maximum possible number of organisms present. A simple method for bioburden determination would not only make feasible routine examination of finished products prior to sterilization, but would allow industry to monitor its manufacturing processes for major sources of contamination with the eventual aim of modifying production lines so as to minimize product contamination, with a concomitant reduction in sterilization costs. In Sweden some manufacturing firms have specifically redesigned their processes to reduce contamination during production (3).

Determination of pre-sterilization bioburden is not, as yet, a compulsory testing procedure, so there is limited available information on procedures and techniques. As part of a service to a local industry we undertook to develop a method for determining bioburden on disposable surgical gloves. To avoid the problem of taking representative samples from an irregularly shaped object, it was decided to utilize a complete glove for each sample.

This study was divided into two parts: development of an adequate washing procedure and selection of a simple method of enumeration.

Initially two washing procedures were considered: use of a Waring blender or of a flask and rotary shaker. The initial studies were conducted with 6-cm^2 pieces of sterile disposable surgical gloves, equally contaminated by soaking in a 48-h *Pseudomonas aeruginosa* culture. The wash solution in all experiments consisted of 0.85% saline with 1.0% Tween 80 added to disperse the powdered coatings on the gloves. Pieces of glove material were washed either in a sterile Waring blender in 100 ml of wash solution (1 min at low speed) or on a rotary shaker (250 rpm for 30 min) in 50 ml of wash solution in the presence of glass beads. The wash solutions were then plated on Trypticase soy agar, and the organisms recovered were counted after 48 h of growth at 37°C (Table 1). The results, when subjected to a two-tailed Student's t test with a 95% confidence interval, showed that the flask and shaker was significantly the more effective method of washing surgical glove material.

When uncontaminated glove samples were tried with this method, the total number of colonies obtained per plate was too low for satisfactory enumeration (less than 30 colonies per plate), indicating a need for sampling larger volumes of wash solution. Membrane filtration (0.2-μm filters) of 20 ml of sample according to standard procedures (2), followed by transfer of the

TABLE 1. *Determination of washing method*

Method	Total count[a] per trial × 10^8	Avg count × 10^8
Waring blender (100 ml of wash solution)	7.0	9.5 ± 3.4
	6.2	
	12.4	
	12.4	
	12.0	
	7.0	
	18.0	
	10.0	
	30.0	
	10.0	
	0.0	
Shaker flask (50 ml of wash solution)	10.8	16.3 ± 4.1
	13.8	
	13.8	
	15.8	
	23.0	
	18.0	
	15.5	
	19.5	
	10.0	
	25.0	
	20.0	
	30.0	

[a] Each number is the average of four plates (plates with between 30 and 300 colony-forming units) made with wash solution from a 6-cm^2 piece of precontaminated glove material.

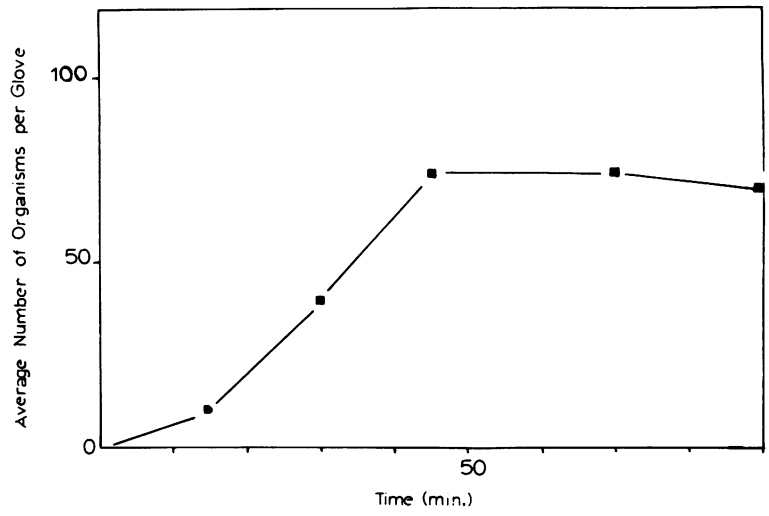

FIG. 1. *Determination of shaker time at 250 rpm on a rotary shaker. Glove material was suspended in 50 ml of wash solution in a 250-ml Erlenmeyer flask.*

filter to 50-mm petri plates of Trypticase soy agar containing 40 mg of actidione per ml (for bacterial counts) or acidified potato dextrose agar (for fungal counts), proved to be a satisfactory alternative.

Nonsterile surgical gloves, aseptically cut open to expose all surfaces to the wash solution, were washed on a rotary shaker for times of 15 to 90 min. It was found that 45 min was the minimum length of time required to wash the gloves efficiently. A 200-ml volume of the wash solution was found to be the most satisfactory in terms of total recovery with the 45-min shaking time (Fig. 1).

The procedure described here provides a simple method for determining pre-sterilization bioburden on surgical gloves which is efficient in terms of both labor and cost. It may be employed to routinely monitor production lines and can be easily adapted for accurate bioburden measurements on any small, irregularly shaped object.

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